[Chem. Pharm. Bull. 32(7)2730—2735(1984)]

Lignans from Bark of the *Olea* Plants. I

HIROKI TSUKAMOTO, SUEO HISADA, and SANSEI NISHIBE*

Faculty of Pharmaceutical Sciences, Higashi Nippon Gakuen University, Ishikari-Tobetsu, Hokkaido 061-02, Japan

(Received November 4, 1983)

Four new lignans, (+)-1-acetoxypinoresinol [(1*S*,2*R*,5*R*,6*S*)-1-acetoxy-2,6-bis(4-hydroxy-3-methoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane] (**1**), (+)-1-hydroxypinoresinol (**2**), (+)-1-acetoxypinoresinol 4''-*O*-methyl ether (**3**) and (+)-1-hydroxypinoresinol 4''-*O*-methyl ether (**4**), and two known lignans, (−)-olivil (**5**) and (+)-cyclo-olivil (**6**), were isolated from the bark of *Olea europaea* L. (Oleaceae). Their structures were elucidated on the basis of spectroscopic analysis and chemical evidence.

The lignans **1**, **2** and **3** were also isolated from the bark of *Olea africana* MILL. (*Olea europaea* L. subsp. *africana* (MILL.) GREEN), and lignans **5** and **6** from the bark of *Olea capensis* L.

Keywords——*Olea europaea*; *Olea africana*; *Olea capensis*; Oleaceae; lignans; (+)-1-acetoxypinoresinol; (1*S*,2*R*,5*R*,6*S*)-1-acetoxy-2,6-bis(4-hydroxy-3-methoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane; (+)-1-hydroxypinoresinol; (+)-1-acetoxypinoresinol 4''-*O*-methyl ether; (+)-1-hydroxypinoresinol 4''-*O*-methyl ether

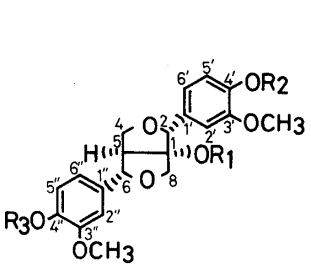
The bark of *Olea europaea* L. (Oleaceae) has been used since olden times as an antifebrile, an anti-rheumatic, a tonic and a remedy for scrofula.[1,2]

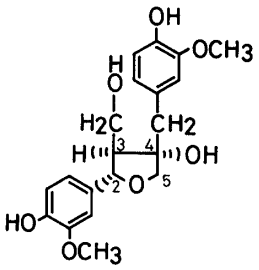
Our interest has been directed to the investigation of the constituents of the bark, with the aim of isolating some biologically active substances, and this paper describes the isolation of four new lignans **1**, **2**, **3** and **4**, along with known lignans, (−)-olivil (**5**) and (+)-cyclo-olivil (**6**), and their structure elucidation on the basis of spectroscopic analysis and chemical evidence.

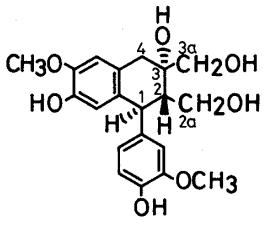
Lignans from the barks of *Olea africana* MILL. and *O. capensis* L. were also investigated. *O. africana* has recently been reclassified as a subspecies of *O. europaea* and is now known as *O. europaea* L. subsp. *africana* (MILL.) GREEN.[3] *O. capensis* is not found outside South Africa.[4] Lignans **1**, **2** and **3** were isolated from *O. africana* and lignans **5** and **6** from *O. capensis*.

The extraction and separation were carried out as described in Experimental. The lignan **1** was obtained as an amorphous powder, $C_{22}H_{24}O_8$, $[\alpha]_D^{21}$ +31.4° (ethanol). The infrared (IR) spectrum of **1** suggested the presence of an ester (1730 cm^{-1}) and aromatic rings (1605 and 1510 cm^{-1}). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** exhibited signals at δ 1.70 (3H, s) due to acetoxy protons, at δ 3.92 (6H, s) due to aromatic methoxy protons and at δ 6.74—7.15 (6H, m) due to aromatic protons. The ultraviolet (UV) spectrum of **1** showed absorption maxima at 233 and 281 nm. The bathochromic shift of the absorption maxima in the presence of base was very similar to that of (+)-pinoresinol.

Acetylation of **1** with acetic anhydride–pyridine gave compound **1a** as a colorless crystalline powder, $C_{26}H_{28}O_{10}$, mp 158—159 °C, $[\alpha]_D^{19}$ +24.7° (chloroform). The ^{1}H-NMR spectrum showed the presence of three acetoxy groups (δ 1.67 and 2.32) and two aromatic methoxy groups (δ 3.88). Methylation of **1** with diazomethane gave compound **1b** as colorless needles, $C_{24}H_{28}O_8$, mp 128—130 °C, $[\alpha]_D^{20}$ +31.8° (chloroform). Deacetylation of **1** with ammonia in methanol gave compound **2**, which was identical with the lignan **2**, isolated as a colorless crystalline powder, $C_{20}H_{22}O_7$, mp 183—185 °C, $[\alpha]_D^{15}$ +39.0° (ethanol). Acetylation







1 : R_1=Ac, R_2=R_3=H
1a: R_1=R_2=R_3=Ac
1b: R_1=Ac, R_2=R_3=CH_3
2 : R_1=R_2=R_3=H
2a: R_1=H, R_2=R_3=Ac
2b: R_1=H, R_2=R_3=CH_3
2c: R_1=H, R_2=R_3=C_2H_5
3 : R_1=Ac, R_2=H, R_3=CH_3
3a: R_1=R_2=Ac, R_3=CH_3
4 : R_1=R_2=H, R_3=CH_3

5

6

Chart 1

of **2** with acetic anhydride–pyridine gave compound **2a** as a colorless crystalline powder, $C_{24}H_{26}O_9$, mp 112—114 °C, $[\alpha]_D^{19}$ +22.0° (chloroform). The ^{1}H-NMR spectrum of **2a** showed the presence of two acetoxy groups (δ 2.33) and two aromatic methoxy groups (δ 3.88). Methylation of **2** with diazomethane gave compound **2b** as colorless plates, $C_{22}H_{26}O_7$, mp 152—153 °C, $[\alpha]_D^{25}$ +27.9° (chloroform); this product was identical with authentic isogmelinol (lit.[5,6] mp 149 °C, $[\alpha]_D$ +30° (chloroform)).

These data disclosed that **1** and **2** have 2,6-diaryl-1-hydroxy-3,7-dioxabicyclo[3.3.0] octane ring. The oxidation of the diethyl ether **2c** with potassium permanganate gave only 4-ethoxy-3-methoxybenzoic acid, suggesting that the aryl groups of **2** are guaiacyl (4-hydroxy-3-methoxyphenyl) units. In the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1**, the downfield shift of the C-1 carbon by 6 ppm relative to that of **2** indicated that an acetoxy group of **1** is attached at the C-1 carbon.

Thus, the structures of **1** and **2** have been established as (+)-1-acetoxypinoresinol [(1*S*, 2*R*, 5*R*, 6*S*)-1-acetoxy-2,6-bis(4-hydroxy-3-methoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane] and (+)-1-hydroxypinoresinol, respectively.

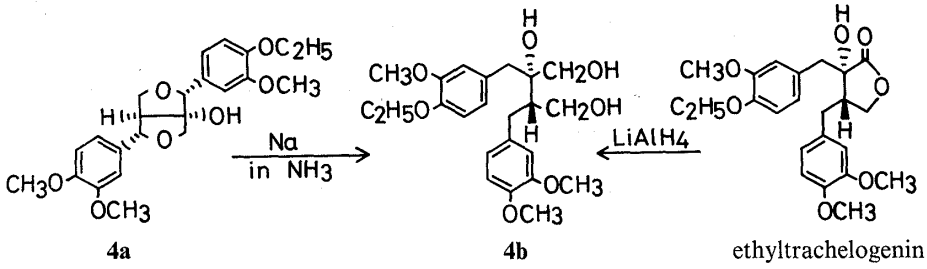
The lignan **3** was obtained as an amorphous powder, $C_{23}H_{26}O_8$, $[\alpha]_D^{23}$ +29.9° (chloroform). The IR spectrum of **3** suggested the presence of an ester (1735 cm^{-1}) and aromatic rings (1600, 1590 and 1510 cm^{-1}). The ^{1}H-NMR spectrum of **3** exhibited signals at δ 1.73 (3H, s) due to acetoxy protons, at δ 3.92 and 3.95 (9H, each s) due to aromatic methoxy protons and at δ 6.72—7.15 (6H, m) due to aromatic protons. The UV spectrum of **3** showed absorption maxima at 233 and 280 nm. The bathochromic shift of the absorption maxima with base was very similar to that of (+)-pinoresinol monomethyl ether. Acetylation of **3** with acetic anhydride–pyridine gave compound **3a** as a colorless crystalline powder, $C_{25}H_{28}O_9$, mp 116—118 °C, $[\alpha]_D^{25}$ +30.5° (chloroform); the ^{1}H-NMR spectrum showed the presence of two acetoxy groups (δ 1.65 and 2.25) and three aromatic methoxy groups (δ 3.79, 3.84 and 3.87). Deacetylation of **3** with ammonia in methanol gave compound **4**, which was identical with the lignan **4**, isolated as an amorphous powder, $C_{21}H_{24}O_7$, $[\alpha]_D^{23}$ +37.9° (chloroform). Methylation of **4** with diazomethane gave **2b**. These data suggested that **3** and **4** bear a marked structural resemblance to **1** and **2**, respectively, and that one of the aryl groups in **3** and **4** is a veratryl (3,4-dimethoxyphenyl) unit.

Table I presents the ^{13}C-NMR data for compounds **1**, **1b**, **2**, **2b**, **3** and **4**, and their

TABLE I. ^{13}C-NMR Chemical Shifts[a)]

	1	1b	2	2b	3	4
C-1	96.9	96.9	91.0	91.0	96.9	91.0
C-5	58.2	58.2	60.8	60.8	58.2	60.8
C-4	69.3	69.4	70.2	70.2	69.3	70.2
C-8	73.9	73.7	74.7	74.7	73.9	74.8
C-2	86.3	86.2	87.1	86.9	86.3	87.1
C-6	84.6	84.2	85.4	85.1	84.4	85.2
C-1′	127.6	129.0	128.1	129.7	127.5	128.0
C-1″	131.2	132.8	132.3	133.9	132.9	134.0
C-2′	113.0	112.4	112.3	111.9	113.0	112.3
C-2″	110.7	110.2	110.8	110.3	110.3	110.3
C-3′	146.9	148.0	146.9	148.1	146.9	146.9
C-3″	147.6	148.7	147.5	148.7	148.8	148.8
C-4′	146.4	148.6	145.9	148.2	146.4	145.9
C-4″		148.3			148.4	148.3
C-5′	114.8	111.1	114.5	111.2	114.9	114.5
C-5″	115.3	111.8	115.1	111.7	111.8	111.7
C-6′	121.3	121.0	120.2	119.7	121.3	120.2
C-6″	118.9	118.4	118.8	118.3	118.5	118.4
OCH_3	55.7	55.5	55.6	55.4	55.5	55.6
					55.7	
$\underline{C}H_3CO$	20.5	20.5			20.5	
$CH_3\underline{C}O$	168.7	168.6			168.7	

a) The spectra were taken in micro cells with a JNM-FX 60 spectrometer (15.00 MHz) in DMSO-d_6 with TMS as an internal reference.



4a **4b** ethyltrachelogenin

Chart 2

assignments. The chemical shifts of the C-1′ carbon in these compounds are around 4 ppm upfield relative to the corresponding signal of the C-1″ carbon. These upfield shifts are due to the γ substituent effect of the alcoholic hydroxyl group at the C-1 carbon. In addition, in (+)-pinoresinol and its dimethyl ether, the chemical shifts of the aromatic C-1, -3, -4 and -5 carbons of the veratryl group are respectively around 1.6, 1.3, 2.4 ppm downfield and 3.5 ppm upfield relative to the corresponding signals of the aromatic carbons of the guaiacyl group, and signals of the aromatic C-2 and C-6 carbons are almost unchanged.[7)] Therefore, the difference of chemical shifts at the C-1″, -3″, -4″ and -5″ carbons between **1** and **3** ($\Delta\delta$ +1.7, +1.2, +2.0 and −3.5), or between **2** and **4** ($\Delta\delta$ +1.7, +1.3, +2.4 and −3.4), clearly indicated that the aryl group at the C-6 position of both **3** and **4** is the veratryl unit.

Thus, the structures of **3** and **4** have been proposed as (+)-1-acetoxypinoresinol 4″-*O*-methyl ether and (+)-1-hydroxypinoresinol 4″-*O*-methyl ether, respectively.

Chemical evidence for these structures was also obtained as follows. The sodium-ammonia reduction of the ethyl ether **4a**, colorless plates, $C_{23}H_{28}O_7$, mp 163—165 °C, $[\alpha]_D^{23}$

+30.5° (chloroform), afforded compound **4b** as a colorless syrup, $C_{23}H_{32}O_7$, $[\alpha]_D^{25}$ −14.2° (chloroform). All the spectral data of **4b** were in good agreement with those of the triol **4b**, which was obtained by the reduction of ethyltrachelogenin[8] with lithium aluminum hydride.

Lignans **5** and **6** were identified as (−)-olivil and (+)-cyclo-olivil, respectively, by direct comparison with authentic samples.

As regards biological activity, it has been reported that the lignans **1** and **3** show high inhibitory activity against cyclic adenosine monophosphate (cAMP)-phosphodiesterase *in vitro* (IC_{50} ($\times 10^{-5}$ M): 3.2 and 11.5).[9]

Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. The following instruments were used: optical rotation, Yanaco OR-50D; UV spectra, Shimadzu UV-210; IR spectra, Shimadzu IR-400; circular dichroism (CD) curves, Jasco J-40; ^{1}H-NMR spectra, Hitachi R-40 with tetramethylsilane ($\delta=0$) as an internal reference; ^{13}C-NMR spectra, JEOL JNM-FX 60, equipped with a JEC-980 computer; mass spectrum (MS), Hitachi RMU-7L and Shimadzu LKB-9000. The abbreviations used are as follows: s, singlet; d, doublet; m, multiplet; br s, broad singlet; t, triplet; q, quartet; sh, shoulder.

Precoated thin-layer chromatography (TLC) plates, Silica gel 60 F_{254} (Merck), were used for TLC and preparative TLC. The spots were detected by spraying the plates with 10% H_2SO_4 soln. and heating. Silica gel (100 mesh, Mallinckrodt) was used for column chromatography.

Isolation——Dry powdered bark of *Olea europaea* (4.0 kg) collected in December 1979 at Shodoshima island, Japan, was extracted four times with hot MeOH. The MeOH solution was evaporated to a small volume under reduced pressure, diluted with water and filtered.

The filtrate was extracted successively with ether, $CHCl_3$ and BuOH. The ether layer was concentrated to dryness. The ether extract (40.0 g) was subjected to column chromatography, eluting with a $CHCl_3$–AcOEt solvent system with gradually increasing proportions of AcOEt. The fractions were monitored by TLC developed with $CHCl_3$–AcOEt (1 : 2). The fractions (100 ml each) showing a TLC spot at *Rf* 0.58 were concentrated. The residue was purified by preparative TLC using $CHCl_3$–AcOEt (1 : 1) and recrystallized from MeOH to give 203.1 mg of **1**. When treated in the same way as described for **1**, the fractions showing TLC spots at *Rf* 0.37, 0.64, 0.46, 0.19 and 0.09 gave 553.6 mg of **2**, 185.4 mg of **3**, 92.6 mg of **4**, 60.0 mg of **5** and 34.8 mg of **6**, respectively.

Dry powdered bark (1.0 kg) of *Olea africana* collected in October 1982 at Bloemfontein, Republic of South Africa, was treated in the same manner as described for *Olea europaea*. The ether extract (5.9 g) gave 23 mg of **1**, 28 mg of **2** and 18 mg of **3**.

Dry powdered bark (110 g) of *olea capensis* collected in November 1982 at Cape Town, Republic of South Africa, was treated in the same manner as described for *Olea europaea*. The ether extract (0.9 g) gave 12 mg of **5** and 7 mg of **6**.

(+)-1-Acetoxypinoresinol (1)——Amorphous powder. $[\alpha]_D^{21}$ +31.4° ($c=0.99$ in EtOH). UV λ_{max}^{EtOH} nm (log ε): 233 (4.18), 281 (3.78). UV $\lambda_{max}^{EtOH+NaOH}$ nm: 255.5, 294. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540 (OH), 1730 (C=O), 1605, 1510 (arom. C=C). MS: Calcd for $C_{22}H_{24}O_8$, 416.1470. Obsd., 416.1474. CD ($c=5.082\times10^{-4}$, ethanol) $[\theta]^{20}\times10^{-3}$ (nm): +1.72 (282), +5.90 (244). ^{1}H-NMR (in $CDCl_3$) δ: 1.70 (3H, s, alcoholic $OCOCH_3$), 3.00—3.57 (1H, m, C_5–H), 3.92 (6H, s, $2\times OCH_3$), 3.65—4.55 (3H, m, $C_{4,8}$–H), 4.36 (1H, dd, $J=8$ and 9 Hz, C_{4e}–H), 4.77 (1H, d, $J=5$ Hz, C_6–H), 5.08 (1H, s, C_2–H), 5.85 (2H, br s, $2\times OH$, quenched by addition of D_2O), 6.74—7.15 (6H, m, arom. H).

(+)-1-Acetoxypinoresinol Diacetate (1a)——**1** (94 mg) was acetylated with acetic anhydride–pyridine in the usual way. The crude acetate was purified by preparative TLC using $CHCl_3$–AcOEt (2 : 1) to give **1a** (64 mg) as a colorless crystalline powder, mp 158—159 °C. $[\alpha]_D^{19}$ +24.7° ($c=0.94$ in $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 218 (4.20), 275 (3.69), 279.8 (3.68). IR ν_{max}^{KBr} cm^{-1}: 1760 (C=O), 1600, 1505 (arom. C=C). MS *m/z*: 500 (M^+, $C_{26}H_{28}O_{10}$). ^{1}H-NMR (in $CDCl_3$) δ: 1.67 (3H, s, alcoholic $OCOCH_3$), 2.32 (6H, s, $2\times$ phenolic $OCOCH_3$), 3.20—3.50 (1H, m, C_5–H), 3.63—4.75 (4H, m, $C_{4,8}$–H), 3.88 (6H, s, $2\times OCH_3$), 4.85 (1H, d, $J=5$ Hz, C_6–H), 5.13 (1H, s, C_2–H), 6.85—7.23 (6H, m, arom. H).

(+)-1-Acetoxypinoresinol Dimethyl Ether (1b)——**1** (178.2 mg) was methylated with diazomethane in the usual way. The crude product was purified by preparative TLC using $CHCl_3$–AcOEt (1 : 1) and recrystallized from EtOH to give **1b** (118.2 mg) as colorless needles, mp 128—130 °C. $[\alpha]_D^{20}$ +31.8° ($c=0.93$ in $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 233 (4.31), 279 (3.81). IR ν_{max}^{KBr} cm^{-1}: 1730 (C=O), 1590, 1510 (arom. C=C). *Anal.* Calcd for $C_{24}H_{28}O_8$: C, 64.85; H, 6.35. Found: C, 64.69; H, 6.33. CD ($c=4.293\times10^{-4}$, ethanol) $[\theta]^{20}\times10^{-3}$ (nm): +1.80 (276), +4.19 (243). ^{1}H-NMR (in $CDCl_3$) δ: 1.70 (3H, s, alcoholic $OCOCH_3$), 3.17—3.49 (1H, m, C_5–H), 3.50—4.70 (4H, m, $C_{4,8}$–H), 3.90 (12H, s, $4\times OCH_3$), 4.82 (1H, d, $J=5$ Hz, C_6–H), 5.10 (1H, s, C_2–H), 6.50—7.15 (6H, m, arom. H).

Deacetylation of (+)-1-Acetoxypinoresinol (1) with Ammonia in Methanol——**1** was deacetylated with ammonia in methanol in the usual way. The crude product was purified by preparative TLC using $CHCl_3$–AcOEt (1 : 2). The

product was identical with the lignan **2**.

(+)-1-Hydroxypinoresinol (2)——Colorless crystalline powder, mp 183—185 °C $[\alpha]_D^{15}$ +39.0° (c=0.65 in EtOH). UV λ_{max}^{EtOH} nm (log ε): 232 (4.27), 281 (3.88). UV $\lambda_{max}^{EtOH+NaOH}$ nm: 253, 293.3. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540 (OH), 1605, 1510 (arom. C=C). MS: Calcd for $C_{20}H_{22}O_7$, 374.1364. Obsd., 374.1367. CD ($c=1.458\times10^{-4}$, ethanol) $[\theta]^{20}\times10^{-3}$ (nm): +1.03 (277), −2.91 (238), +8.22 (212). ^{1}H-NMR (in $CDCl_3$) δ: 3.00—3.23 (1H, m, C_5–H), 3.87 (6H, s, 2×OCH_3), 3.65—4.22 (3H, m, $C_{4,8}$–H), 4.53 (1H, dd, J=8 and 9 Hz, C_{4e}–H), 4.80 (1H, s, C_2–H), 4.85 (1H, d, J=5 Hz, C_6–H), 6.67—7.10 (6H, m, arom. H).

(+)-1-Hydroxypinoresinol Diacetate (2a)——**2** (40 mg) was acetylated with acetic anhydride–pyridine in the usual way. The crude acetate was purified by preparative TLC using $CHCl_3$–AcOEt (2:1) to give **2a** (30 mg) as a colorless crystalline powder, mp 112—114 °C. $[\alpha]_D^{19}$ +22.0° (c=0.45 in $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 218.2 (4.26), 273.8 (3.74), 279.2 (3.72). IR ν_{max}^{KBr} cm^{-1}: 3415 (OH), 1755 (C=O), 1605, 1510 (arom. C=C). MS m/z: 458 (M^+, $C_{24}H_{26}O_9$). ^{1}H-NMR (in $CDCl_3$) δ: 2.33 (6H, s, 2× phenolic $OCOCH_3$), 2.97—3.33 (1H, m, C_5–H), 3.75—4.26 (3H, m, $C_{4,8}$–H), 3.88 (6H, s, 2×OCH_3), 4.59 (1H, dd, J=8 and 9 Hz, C_{4e}–H), 4.89 (1H, s, C_2–H), 4.94 (1H, d, J=5 Hz, C_6–H), 6.86—7.29 (6H, m, arom. H).

(+)-1-Hydroxypinoresinol Dimethyl Ether (2b)——**2** (45 mg) was methylated with diazomethane in the usual way. The crude product was purified by preparative TLC using $CHCl_3$–AcOEt (1:1) and recrystallized from EtOH to give **2b** (34.5 mg) as colorless plates, mp 152—153 °C. $[\alpha]_D^{25}$ +27.9° (c=0.57 in $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 232 (4.28), 278.7 (3.78). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1600, 1585, 1510 (arom. C=C). *Anal.* Calcd for $C_{22}H_{26}O_7$: C, 65.66; H, 6.51. Found: C, 65.61; H, 6.54. CD ($c=5.531\times10^{-4}$, ethanol) $[\theta]^{20}\times10^{-3}$ (nm): +0.93 (277.5), −1.06 (244), +0.31 (228), −0.14 (215). ^{1}H-NMR (in $CDCl_3$) δ: 1.73 (1H, s, OH, quenched by addition of D_2O), 2.80—3.35 (1H, m, C_5–H), 3.91 (12H, s, 4×OCH_3), 3.50—4.36 (3H, m, $C_{4,8}$–H), 4.57 (1H, dd, J=8 and 9 Hz, C_{4e}–H), 4.87 (1H, s, C_2–H), 4.90 (1H, d, J=5 Hz, C_6–H), 6.75—7.17 (6H, m, arom. H).

The properties and spectral data of **2b** were in good agreement with those of isogmelinol given in the literature (mp 149 °C, $[\alpha]_D$ +30° (in $CHCl_3$)).[5,6]

Oxidation of (+)-1-Hydroxypinoresinol Diethyl Ether (2c) with Potassium Permanganate——**2** (30 mg) in EtOH was ethylated with diazoethane to give **2c**. Without purification, **2c** was dissolved in 10 ml of 1 N NaOH soln. with warming on a water bath. The solution was treated with 2% $KMnO_4$ soln. in small portions at 35 °C (pink end point). The color was discharged with sodium bisulfite solution and the precipitate was filtered off. The filtrate, after being acidified with dil. H_2SO_4 soln., was extracted with ether. The ether solution was evaporated to yield the oxidation product as colorless needles, mp 196—197 °C.

This product was identified by direct comparison with authentic 4-ethoxy-3-methoxybenzoic acid.

(+)-1-Acetoxypinoresinol 4″-*O*-Methyl Ether (3)——Amorphous powder. $[\alpha]_D^{23}$ +29.9° (c=1.14 in $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 233 (4.16), 280 (3.67). UV $\lambda_{max}^{EtOH+NaOH}$ nm: 233, 256, 285, 292. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540 (OH), 1735 (C=O), 1600, 1590, 1510 (arom. C=C). MS: Calcd for $C_{23}H_{26}O_8$, 430.1626. Obsd., 430.1611. CD ($c=5.051\times10^{-4}$, ethanol) $[\theta]^{20}\times10^{-3}$ (nm): +1.43 (275), +3.27 (243). ^{1}H-NMR (in $CDCl_3$) δ: 1.73 (3H, s, alcoholic $OCOCH_3$), 3.19—3.47 (1H, m, C_5–H), 3.92, 3.95 (9H, each s, 3×OCH_3), 3.50—4.72 (4H, m, $C_{4,8}$–H), 4.83 (1H, d, J=5 Hz, C_6–H), 5.11 (1H, s, C_2–H), 6.72—7.15 (6H, m, arom. H).

(+)-1-Acetoxypinoresinol 4″-*O*-Methyl Ether Monoacetate (3a)——**3** (55 mg) was acetylated with acetic anhydride–pyridine in the usual way. The crude acetate was purified by preparative TLC using $CHCl_3$–AcOEt (2:1) to give **3a** (53.1 mg) as colorless crystalline powder, mp 116—118 °C. $[\alpha]_D^{25}$ +30.5° (c=0.66 in $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 224.8 (4.18), 279.5 (3.74). IR ν_{max}^{KBr} cm^{-1}: 1760, 1735 (C=O), 1600, 1510 (arom. C=C). MS: Calcd for $C_{25}H_{28}O_9$, 472.1731. Obsd., 472.1735. ^{1}H-NMR (in $CDCl_3$) δ: 1.65 (3H, s, alcoholic $OCOCH_3$), 2.25 (3H, s, phenolic $OCOCH_3$), 3.20—3.50 (1H, m, C_5–H), 3.79, 3.84, 3.87 (9H, each s, 3×OCH_3), 4.15—4.61 (4H, m, $C_{4,8}$–H), 4.74 (1H, d, J=5 Hz, C_6–H), 5.05 (1H, s, C_2–H), 6.68—7.10 (6H, m, arom. H).

Deacetylation of (+)-1-Acetoxypinoresinol 4″-*O*-Methyl Ether (3) with Ammonia in Methanol——**3** was deacetylated with ammonia in methanol in the usual way. The crude product was purified by preparative TLC using $CHCl_3$–AcOEt (1:2). The product was identical with the lignan **4**.

(+)-1-Hydroxypinoresinol 4″-*O*-Methyl Ether (4)——Amorphous powder. $[\alpha]_D^{23}$ +37.9° (c=0.81 in $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 232.5 (4.21), 280 (3.75). UV $\lambda_{max}^{EtOH+NaOH}$ nm: 235, 253, 285, 295. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540 (OH), 1605, 1590, 1510 (arom. C=C). MS: Calcd for $C_{21}H_{24}O_7$, 388.1520. Obsd., 388.1538. CD ($c=4.727\times10^{-4}$, ethanol) $[\theta]^{20}\times10^{-3}$ (nm): +1.06 (278), −0.36 (242.5), +0.32 (240), +0.33 (228). ^{1}H-NMR (in $CDCl_3$) δ: 1.80 (1H, br s, OH, quenched by addition of D_2O), 2.94—3.33 (1H, m, C_5–H), 3.91 (9H, s, 3×OCH_3), 3.42—4.72 (4H, m, $C_{4,8}$–H), 4.83 (1H, s, C_2–H), 4.88 (1H, d, J=5 Hz, C_6–H), 6.59—7.24 (6H, m, arom. H).

Methylation of (+)-1-Hydroxypinoresinol 4″-*O*-Methyl Ether (4)——**4** in MeOH was methylated with diazomethane in the usual way. The crude product was purified by preparative TLC using $CHCl_3$–AcOEt (1:1). The product was identical with **2b**.

(+)-1-Hydroxypinoresinol 4′-*O*-Ethyl-4″-*O*-methyl Ether (4a)——**4** (40 mg) in EtOH was treated with diazoethane in the usual way. The crude product was purified by preparative TLC using $CHCl_3$–AcOEt (1:1) and recrystallized from EtOH to give **4a** (30 mg) as colorless plates, mp 163—165 °C. $[\alpha]_D^{22}$ +30.5° (c=0.50 in $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 232.5 (4.31), 320 (3.81). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560 (OH), 1610, 1600, 1520 (arom. C=C). MS: Calcd

for $C_{23}H_{28}O_7$, 416.1833. Obsd., 416.1838. ^{1}H-NMR (in $CDCl_3$) δ: 1.47 (3H, t, J=8 Hz, $OCH_2C\underline{H}_3$), 1.75 (1H, br s, OH, quenched by addition of D_2O), 3.65—4.36 (3H, m, $C_{4,8}$–H), 3.92 (9H, s, 3×OCH_3), 4.02 (2H, q, J=8 Hz, $OC\underline{H}_2CH_3$), 4.58 (1H, dd, J=8 and 9 Hz, C_{4e}–H), 4.87 (1H, s, C_2–H), 4.92 (1H, d, J=5 Hz, C_6–H), 6.85—7.20 (6H, m, arom. H).

Sodium-Ammonia Reduction of (+)-1-Hydroxypinoresinol 4′-*O*-Ethyl-4″-*O*-methyl Ether (4a)——**4a** (30 mg) in pure tetrahydrofuran (THF) (6 ml) was added to liquid ammonia, and the stirred solution was treated with sodium (50 mg). After the disappearance of the blue color, a little water was added to the yellow solution and the ammonia was evaporated off. The residue was treated with H_2O, the mixture was extracted with $CHCl_3$, and the extract was concentrated. The residue was purified by preparative TLC using $CHCl_3$–AcOEt (1 : 1) to give **4b** as a colorless syrup. $[\alpha]_D^{25}$ −14.2° (c=1.0 in $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 230 (4.20), 280.5 (3.74). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560, 3400 (OH), 1590, 1515 (arom. C=C). MS: Calcd for $C_{23}H_{32}O_7$, 420.2145. Obsd., 420.2125. MS m/z: 420 (M^+, $C_{23}H_{32}O_7$, 11.7%), 254 ($C_{13}H_{18}O_5$, 15.8%), 237 ($C_{13}H_{17}O_4$, 12.3%), 166 ($C_{10}H_{14}O_2$, 87.1%), 151 ($C_9H_{11}O_2$, 100%), 137 ($C_8H_9O_2$, 32.2%). ^{1}H-NMR (in $CDCl_3$) δ: 1.46 (3H, t, J=8 Hz, $OCH_2C\underline{H}_3$), 1.90—2.29 (1H, m, C_3–H), 2.47—2.63 (2H, m, C_4–H), 2.94 (2H, br s, C_1–H), 3.28—3.78 (4H, m, $C_{2a,3a}$–H), 3.53 (2H, br s, 2×OH, quenched by addition of D_2O), 3.85 (9H, s, 3×OCH_3), 3.96 (2H, q, J=8 Hz, $OC\underline{H}_2CH_3$), 6.57—7.12 (6H, m, arom. H).

Reduction of Ethyltrachelogenin with Lithium Aluminum Hydride——A solution of ethyltrachelogenin[8] (130.9 mg) in THF (5 ml) was added dropwise to a suspension of $LiAlH_4$ (260 mg) in THF (30 ml). The mixture was stirred for 8 h at room temperature and then poured into ice-cold water. The whole was carefully acidified with 10% H_2SO_4 soln. and extracted with ether. The ether soln. was washed with water and concentrated *in vacuo*. The residue showed a spot of *Rf* 0.25 on TLC using $CHCl_3$–AcOEt (1 : 1). Purification by TLC afforded the triol **4b** as a colorless syrup.

All the spectral data of the product were in good agreement with those of the triol **4b**.

(−)-Olivil (5)——Colorless needles from EtOH. mp 121—123 °C, $[\alpha]_D^{23}$ −23.9° (c=1.29 in EtOH). UV λ_{max}^{EtOH} nm (log ε): 230.4 (4.10), 281.5 (3.72). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1600, 1510 (arom. C=C). MS m/z: 376 (M^+, $C_{20}H_{24}O_7$). ^{1}H-NMR (in CD_3OD) δ: 2.10—2.83 (1H, m, C_3–H), 2.92 (2H, s, –$C\underline{H}_2$–), 3.76 (6H, s, 2×OCH_3), 3.38—3.98 (2H, m, –$C\underline{H}_2$OH), 3.97—4.50 (2H, m, C_5–H), 6.46—7.15 (6H, m, arom. H).

This was identified by direct comparison with authentic (−)-olivil.

(+)-Cyclo-olivil (6)——Colorless needles from EtOH. mp 168—170 °C, $[\alpha]_D^{23}$ +51.8° (c=0.77 in EtOH). UV λ_{max}^{EtOH} nm (log ε): 230.5 (4.03), 284 (3.69). IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 1620, 1520 (arom. C=C). MS m/z: 376 (M^+, $C_{20}H_{24}O_7$). ^{1}H-NMR (in CD_3OD) δ: 1.90—2.24 (1H, m, C_2–H), 2.60 (1H, d, J=17 Hz, C_4–H), 3.20 (1H, d, J=17 Hz, C_4–H), 3.73, 3.75 (6H, each s, 2×OCH_3), 3.40—4.35 (5H, m, $C_{1,2a,3a}$–H), 6.15—6.85 (5H, m, arom. H).

This was identified by direct comparison with authentic (+)-cyclo-olivil.

Acknowledgement We wish to thank Asst. Prof. S. Yamanouchi, Department of Pharmacy, College of Sciences and Technology, Nihon University, for measurement of MS with a Hitachi RMU-7L spectrometer.

References and Notes

1) F. B. Power and F. Tutin, *J. Chem. Soc.*, **93**, 904 (1908).
2) J. M. Watt and M. G. Breyer-Bramdwijk, "The Medicinal and Poisonous Plants of Southern and Eastern Africa," Edinburgh, Livingstone, 1962, p. 806.
3) P. S. Green and F. K. Kupicha, *Kew Bull.*, **34**, 69 (1979).
4) L. E. W. Codd (ed.), "Flora of Southern Africa," Vol. 26, Botanical Research Institute, Pretoria, 1963, p. 112.
5) R. H. Harradence and F. Lions, *J. Proc. Roy. Soc. New South Wales*, **74**, 117 (1940).
6) A. J. Birch, P. L. Macdonald, and A. Pelter, *J. Chem. Soc. (C)*, **1967**, 1968.
7) Unpublished. We presented the chemical shifts of the C-3 and C-4 carbons of the guaiacyl unit of (+)-pinoresinol as 145.9 and 147.6 ppm and the shift values due to *O*-methylation as +2.4 and +1.3, respectively, at the 26th Symposium on the Chemistry of Natural Products, Kyoto, 1983. Later, in a private communication and paper[10] on the effect of *O*-methylation of *ortho*-substituted phenols from Prof. M. Nagai, Hoshi College of Pharmacy, it was proposed that the assignment should be reversed. In conjunction with Prof. Nagai's group, we have concluded that the assignments for the C-3 and C-4 carbons should in deed be reversed to 147.6 and 145.9 ppm, and the *O*-methylation shift values to +1.3 and +2.4, respectively. A detailed report on these result is in preparation.
8) I. Inagaki, S. Hisada, and S. Nishibe, *Chem. Pharm. Bull.*, **20**, 2710 (1972).
9) T. Nikaido, T. Ohmoto, T. Kinoshita, U. Sankawa, S. Nishibe, and S. Hisada, *Chem. Pharm. Bull.*, **29**, 3586 (1981).
10) M. Fujita, M. Nagai, and T. Inoue, *Chem. Pharm. Bull.*, **30**, 1151 (1982).